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Effects on cultured mammalian cells of myotoxin III, a phospholipase A2 isolated from *Bothrops asper* (terciopelo) venom.

Butron E, Ghelestam M, Gutierrez JM.

Departamento de Bioquimica y Nutricion, Facultad de Medicina, Universidad de Panama, Panama City.

Myotoxin III (MT-III), a myotoxic phospholipase A2 from *Bothrops asper*, was studied with respect to interactions with cultured mammalian cells and red blood cells. Tests of the cytopathogenic effect of MT-III on different cell lines indicated that rat skeletal muscle L6 myoblasts were more sensitive to the toxin than Chinese hamster ovary cells, human lung fibroblasts, mouse adrenal tumour cells and rat intestinal epithelial cells. Specific plasma-membrane permeabilization was assayed as release of a cytosolic [³H]uridine nucleotide marker from toxin-treated L6 cells. A dose- and time-related membrane permeabilization was induced at 37 degrees C, but not at 0 degree C. A half-maximal effect was obtained after 20 min. 30 micrograms/ml MT-III induced 50% marker release in 1 h, and the effect was not reversed by post-incubation for up to 48 h in toxin-free medium. The membrane permeabilization in L6 cells did not seem to require cellular internalisation of the toxin. The catalytic site of the toxin was inactivated by alkylation with p-bromophenacyl bromide (BPB). This treatment abolished the toxin's specific PLA₂ activity, as assayed in vitro, and reduced the PLA₂ activity on the myoblast membrane by more than 95%, as measured by release of [¹⁴C]arachidonic acid from prelabelled cells. However, the membrane-permeabilizing effect (release of cytosolic marker) was reduced only by 70% upon modification with BPB. We also report that MT-III is not directly haemolytic, and one reason for this is the inability of the toxin to associate with the membranes of human or mouse erythrocytes. Taken together, the data suggest that MT-III at 37 degrees C binds to and penetrates the plasma membrane of cultured myoblasts, thereby inducing a rapid, direct and irreversible membrane permeabilization. This effect apparently depends in part on the PLA₂ activity of the toxin and in part on a molecular region which is separate from the catalytic site.

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Recombinant human secretory phospholipase A2: purification and characterization of the enzyme for active site studies.

Stadel JM, Jones C, Livi GP, Hoyle K, Kurdyla J, Roshak A, McLaughlin MM, Pfarr DA, Comer S, Strickler J, et al.

Department of Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

A secreted form of phospholipase A2 (PLA2) is thought to play an important role in inflammatory diseases. To characterize this enzyme the cDNA encoding a low molecular weight PLA2 was cloned from a human placental cDNA library. The cDNA encoding the human PLA2 was subcloned into an expression vector and subsequently transfected into Chinese hamster ovary (CHO) cells. A stable CHO cell clone, secreting ca 1 mg/L of recombinant PLA2 into the medium, was scaled up in culture to 180 L. The recombinant enzyme was purified from the cell supernatant to apparent homogeneity by a novel procedure combining adsorption to poly(vinylidene difluoride) membranes, ion exchange chromatography and size exclusion chromatography. The final recovery of PLA2 activity was 58%. A direct comparison between the purified recombinant human PLA2 and PLA2 purified from human synovial fluid, including molecular weight, antigenicity, ionic dependence, substrate specificity and sensitivity to known PLA2 inhibitors, indicated that the two enzymes exhibit identical biochemical properties. These results show that the recombinant PLA2 can be efficiently expressed and purified in sufficient quantities to characterize the enzyme active site, to aid in the rational development of PLA2 inhibitors as potential anti-inflammatory drugs, and to investigate further the role of PLA2 in inflammatory disease.

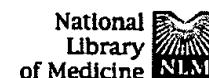
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An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma.

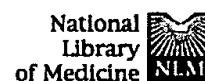
Stremler KE, Stafforini DM, Prescott SM, Zimmerman GA, McIntyre TM.

Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City 84112.

Platelet-activating factor (PAF) is a glycerophospholipid that has diverse potent biological actions. A plasma enzyme catalyzes the hydrolysis of the sn-2 acetyl group of PAF and thereby abolishes its bioactivity. This PAF acetylhydrolase is specific for phospholipids, such as PAF, with a short acyl group at the sn-2 position. The majority of it (60-70%) is associated with low density lipoprotein (LDL), and the remainder is with high density lipoprotein (HDL). LDL also has a phospholipase A2 activity that is specific for oxidized polyunsaturated fatty acids, which may be important in determining how LDL is recognized by cellular receptors. We previously have purified and characterized the PAF acetylhydrolase from human plasma. We now have found that the purified PAF acetylhydrolase catalyzes the hydrolysis of the oxidized fragments of arachidonic acid from the sn-2 position of phosphatidylcholine. One of the preferred substrates appeared by mass spectrometry to have 5-oxovalerate at the sn-2 position. We synthesized 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine and found that the PAF acetylhydrolase had the same apparent K_m for it (11.3 microM) as for PAF (12.5 microM), with V_{max} values of 100 and 167 $\mu\text{mol}/\text{h}/\text{mg}$ of protein, respectively. We also conclude that the PAF acetylhydrolase is the sole activity in LDL that degrades oxidized phospholipids since we found co-localization of the activity against both substrates to LDL and HDL, and precipitation of enzyme activity with an antibody to the PAF acetylhydrolase. Thus, the PAF acetylhydrolase in human plasma degrades oxidized phospholipids, which may be involved in the modification of apolipoprotein B100 and other pathological processes.

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Hydrolysis of phosphatidylcholine during LDL oxidation is mediated by platelet-activating factor acetylhydrolase.

Steinbrecher UP, Pritchard PH.

Department of Medicine, University of British Columbia, Vancouver, Canada.

Degradation of phosphatidylcholine to lysophosphatidylcholine occurs during oxidative modification of low density lipoproteins (LDL). In this study, we have shown that this phospholipid hydrolysis is brought about by an LDL-associated phospholipase A2 that can hydrolyze oxidized but not intact LDL phosphatidylcholine. The chemical nature of the oxidized phospholipids that can act as substrates for this enzyme was not fully characterized, but we hypothesized that the specificity of the enzyme for oxidized LDL phosphatidylcholine might be explained by fragmentation of polyunsaturated sn-2 fatty acyl groups in LDL phosphatidylcholine during oxidation. To facilitate characterization of this enzyme, we therefore selected a fluorescent phosphatidylcholine substrate that had a short-chain, polar residue in the sn-2 position: 1-palmitoyl 2-(6-[7-nitrobenzoxadiazolyl]amino) caproyl phosphatidylcholine, (C6NBD PC). This substrate was efficiently hydrolyzed by LDL, but the dodecanoyl analogue of C6NBD PC, which differed only in that a 12-carbon rather than a 6-carbon acyl derivative was present in the sn-2 position, was not hydrolyzed. The phospholipase activity was heat-stable, calcium-independent, and was inhibited by the serine esterase inhibitors phenylmethylsulfonyl-fluoride and diisopropylfluorophosphate, but was resistant to p-bromophenacylbromide and dithiobisnitrobenzoic acid. The phospholipid hydrolysis could not be attributed to the action of lecithin:cholesterol acyltransferase or lipoprotein lipase. Nearly all of the activity in EDTA-anticoagulated normal plasma was physically associated with apoB-containing lipoproteins, but this apoprotein was not essential as enzyme activity was present in plasma from abetalipoproteinemic patients. These properties are very similar to those recently reported for human plasma platelet-activating factor (PAF) acetylhydrolase. In the present study, we found that acylhydrolase activity against C6NBD PC, PAF, and oxidized phosphatidylcholine copurified through gel filtration and ion-exchange chromatography. Substrate competition was demonstrated between C6NBD PC, PAF, and oxidized 2-arachidonoyl phosphatidylcholine, suggesting that a single enzyme was active against all three substrates. The enzyme had an apparent .

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molecular weight of 40,000-45,000 by high pressure gel exclusion chromatography. Inhibition of this activity with disopropylfluorophosphate prior to oxidative modification of LDL prevented phospholipid hydrolysis but did not affect the production of thiobarbituric acid reactive compounds or the change in electrophoretic mobility. In addition, this inhibition of phospholipase did not prevent the rapid degradati

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